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Antimicrobial Resistance-Conferring Plasmids with Similarity to Virulence Plasmids from Avian Pathogenic *Escherichia coli* Strains in *Salmonella enterica* Serovar Kentucky Isolates from Poultry▽†

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***Salmonella enterica*, a leading cause of food-borne gastroenteritis worldwide, may be found in any raw food of animal, vegetable, or fruit origin. *Salmonella* serovars differ in distribution, virulence, and host specificity. *Salmonella enterica* serovar Kentucky, though often found in the food supply, is less commonly isolated from ill humans. The multidrug-resistant isolate *S.* Kentucky CVM29188, isolated from a chicken breast sample in 2003, contains three plasmids (146,811 bp, 101,461 bp, and 46,121 bp), two of which carry resistance determinants (pCVM29188_146 [*strAB* and *tetRA*] and pCVM29188_101 [bla_{CMY-2} and *sugE*]). Both resistance plasmids were transferable by conjugation, alone or in combination, to *S.* Kentucky, *Salmonella enterica* serovar Newport, and *Escherichia coli* recipients. pCVM29188_146 shares a highly conserved plasmid backbone of 106 kb (>90% nucleotide identity) with two virulence plasmids from *a*vian *p*athogenic *Escherichia coli* strains (pAPEC-O1-ColBM and pAPEC-O2-ColV). Shared avian pathogenic *E. coli* (APEC) virulence factors include *iutA iucABCD*, *sitABCD*, *etsABC*, *iss*, and *iroBCDEN*. PCR analyses of recent (1997 to 2005) *S.* Kentucky isolates from food animal, retail meat, and human sources revealed that 172 (60%) contained similar APEC-like plasmid backbones. Notably, though rare in human- and cattle-derived isolates, this plasmid backbone was found at a high frequency (50 to 100%) among *S.* Kentucky isolates from chickens within the same time span. Ninety-four percent of the APEC-positive isolates showed resistance to tetracycline and streptomycin. Together, our findings of a resistance-conferring APEC virulence plasmid in a poultry-derived *S.* Kentucky isolate and of similar resistance/virulence plasmids in most recent *S.* Kentucky isolates from chickens and, to lesser degree, from humans and cattle highlight the need for additional research in order to examine the prevalence and spread of combined virulence and resistance plasmids in bacteria in agricultural, environmental, and clinical settings.**

Nontyphoidal *Salmonella enterica* infections are one of the leading causes of bacterial food-borne gastroenteritis worldwide and an important public health problem in the United States, causing an estimated 1.4 million cases of infection, 15,000 hospitalizations, and more than 400 deaths annually in the United States alone (41). Although *Salmonella* infection usually presents with self-limiting diarrhea, in some patient populations, such as the immunocompromised, it can lead to life-threatening systemic infections that require effective and immediate antimicrobial therapy (21). The global emergence of multidrug resistance in *S. enterica* isolates from agricultural and clinical settings has therefore raised concerns and resulted in the establishment of several national resistance surveillance programs, such as the European Antimicrobial Resistance Surveillance System and the National Antimicrobial Monitoring System (NARMS) in the United States.

In the United States, antimicrobial compounds are widely used in agriculture, not only to treat and prevent disease in plants, fruits, vegetables, and animals but also to promote growth in poultry and other livestock (25, 38). As a consequence, multidrug resistance is commonly detected in enterobacteria isolated from veterinary sources, including nontyphoidal *Salmonella* and other food-borne pathogens (40). Several studies have indicated the possibility that resistance reservoirs in animals can promote the transmission of resistance determinants from agricultural to clinical settings via food contaminants (1, 2, 45). Whether antimicrobial use in agriculture enhances the distribution and spectrum of antimicrobial resistance phenotypes in clinical settings has been the focus of vigorous debate within the public health and research communities (15, 19, 24, 27, 43). The extent of multidrug resistance in

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food-borne pathogens (10, 40), however, remains a concern. In 2006, only 17.7%, 25.0%, 38.8%, and 73.7% of the nontyphoidal *Salmonella* isolates from ground turkey, pork chop, chicken breast, and ground beef samples, respectively, showed susceptibility to all 16 antimicrobial compounds tested as part of the NARMS program (10). On the other hand, the overall prevalence of antimicrobial resistance phenotypes in nontyphoidal *Salmonella* isolates from human sources has slightly decreased, from 33.8% of all 876 isolates tested in 1996 to 19.4% of all 1,654 isolates tested in 2005 displaying a detectable resistance phenotype to at least one out of five antimicrobial subclasses as defined by the Clinical and Laboratory Standards Institute. However, in the same interval, increases from 0.4% to 2.4% and 0.2% to 2.9% in resistance to the clinically important subclasses of quinolones (nalidixic acid [Nal]) and cephalosporins (ceftiofur), respectively, were observed for the same set of human *Salmonella* isolates (6). Altogether, these reports demonstrate the need for further investigations on the influence of antimicrobial selection on the evolution, distribution, and transmission of resistance and virulence phenotypes among enteric bacteria derived from agricultural and clinical settings in order to prevent or at least limit the future spread of resistant zoonotic pathogens between these environments.

Salmonella enterica subsp. *enterica* serovar Kentucky is widespread in poultry meats but is relatively uncommon in human cases of salmonellosis (7). *S.* Kentucky did not rank among the 20 most frequent *Salmonella* serotypes isolated from human sources in 2006 (7). In food-related sources, however, it is often found in animal samples and has been the most common serotype isolated from chickens (48.8%) (40) and chicken meat (38.8%) (10). At lower proportions, it is also present in turkey and cattle (2.6% and 3.6% of all nontyphoidal *Salmonella* isolates, respectively) (40). In the past decade, the fraction of *S.* Kentucky isolates from chickens, compared with other serotypes, has been increasing steadily, from 25% in 1997 to almost 50% in 2006 (40). Interestingly, while on average only 63 (0.174%) *S.* Kentucky isolates were reported between 1996 and 2004 among all nontyphoidal salmonellae from human samples, this number increased in 2005 (81 isolates [0.224%]) and 2006 (123 isolates [0.302%]) (7). Antimicrobial resistance phenotypes in *S.* Kentucky isolates from chicken meat are overrepresented compared to those in other serovars, with resistance to tetracycline (72.9%) and streptomycin (69.5%) being most commonly found (10). Although a causal connection between the increase of *S.* Kentucky in chickens and the number of human infections caused by the same serovar has not been demonstrated, further investigation is warranted, particularly in light of the high prevalence of antimicrobial resistance phenotypes in *S.* Kentucky isolates from chickens and the increasing resistance to beta-lactam compounds.

Here, we describe the plasmid component of the genome of *S.* Kentucky CVM29188, a multidrug-resistant strain that was isolated in 2003 from a chicken meat sample with resistance to streptomycin, tetracycline, ampicillin, and ceftiofur. Using a combination of in silico and in vivo approaches, including comparative plasmid sequence analysis, conjugative plasmid transfer, and PCR-based plasmid screenings, we present new insights into the genetic basis for multidrug resistance phenotypes of this isolate that provide new clues about virulence evolution and host adaptation in this *Salmonella* serovar.

MATERIALS AND METHODS

Bacterial strains and PCR primers. *Salmonella enterica* subsp. *enterica* serovar Kentucky strain CVM29188 was isolated in 2003 from a sample of chicken breast purchased through the NARMS retail meat surveillance program in Georgia. According to Clinical and Laboratory Standards Institute guidelines, *S.* Kentucky CVM29188 was determined to be resistant to streptomycin (MIC, >64 μg/ml), tetracycline (MIC, >32 μg/ml), ampicillin (MIC, >32 μg/ml), and ceftiofur (MIC, >8 μg/ml). For mating experiments, the plasmid-free *S.* Kentucky strain CVM35942, isolated from a human sample in 2002; *Salmonella enterica* subsp. *enterica* serovar Newport strain SL317, isolated from a human sample in 2002; and the laboratory *Escherichia coli* strain DH10B were used, none of which have known antimicrobial resistance phenotypes or carry plasmids. The PCR primers used for the verification of plasmid transconjugants and for the plasmid screening assay are listed in Table S1 in the supplemental material. All *Salmonella* strains that were screened for APEC-like plasmids similar to pCVM29188_146 (see Results for details) were isolated between 1997 and 2007 and selected from strain collections maintained through the NARMS at the Centers for Disease Control and Prevention (CDC) National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Atlanta, GA (human isolates); the U.S. Department of Agriculture Agricultural Research Service, Russell Research Center, Athens, GA (animal isolates); and the Food and Drug Administration Center for Veterinary Medicine (food isolates). A complete list of all isolates, together with plasmid typing results, antimicrobial susceptibility patterns, and source information, is given in Table S2 in the supplemental material.

Genome sequencing and annotation. Whole-genome, random shotgun plasmid insert libraries of 3 to 5 kbp and 10 to 12 kbp and fosmid insert libraries of 30 to 40 kbp were constructed as previously reported (28) and sequenced using a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA). Assembly and closure were performed as previously described (28). Plasmid sequence comparisons were carried out with the NUCmer program, which is part of the MUMmer package (20). The prediction of horizontally transferred genes based on codon usage analysis was performed with the SIGI-HMM tool (42).

Plasmid mating experiments. Conjugative plasmid transfer systems with the strains described above were set up as described previously (44). Briefly, rifampin (rifampicin) (Rif)- and Nal-resistant recipient strains were generated from 1-ml overnight cultures by streaking the concentrated cells from 1 ml onto LB-Rif (100 μg/ml) or LB-Nal (10 μg/ml) plates, respectively, and by picking resistant clones after 24 h of incubation. For mating reactions, donor and recipient strains were grown in an orbital shaker (225 rpm) in LB medium at 37°C to mid-log phase. Donor and recipient bacteria were then harvested by centrifugation (16,100 × *g*, 1 min), and 10^8 CFU of donor and recipient was mixed and spotted onto LB agar plates. Conjugation mixtures were allowed to incubate for 3 h at 37°C, after which the cells were resuspended in phosphate-buffered saline, diluted, and plated onto LB agar containing the appropriate medium to counterselect for the donor and the resistance marker encoded by the plasmid(s), i.e., either tetracycline (10 μg/ml) or ceftiofur (2 μg/ml) or both agents in combination. Transconjugants were assayed for the presence of the plasmid-specific *repA* marker genes (pCVM29188_146 [IncFIB], pCVM29188_101 [IncI1], and pCVM29188_46 [IncFII]) using PCR.

PCR plasmid screening. PCR-based screening assays were carried out to test *S.* Kentucky isolates from different sources, as well as isolates from several other nontyphoidal *Salmonella* serotypes, for the presence of APEC-like plasmids similar to pCVM29188_146. Plasmid DNA for PCR was prepared using a QIAprep Spin miniprep kit according to the manufacturer's procedure (Qiagen, Valencia, CA). PCR was performed using PTC-200 (MJ Research, Waltham, MA) with the following cycling protocol: initial denaturation (5 min at 94°C); followed by 35 cycles of denaturation (60s at 94°C), annealing (60s at 62°C), and elongation (90s at 72°C); and the final extension (10 min at 72°C). The five primer sets used were each designed to amplify a distinct plasmid backbone region displayed in Fig. 1. Primers are listed in Table S1 in the supplemental material.

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed to determine genomic DNA fingerprinting profiles of *Salmonella* serovar Kentucky according to the protocol developed by the CDC (29). Agarose-embedded DNA was digested with 50 U of XbaI (Boehringer Mannheim, Indianapolis, IN) for at least 4 h in a water bath at 37°C. The restriction fragments were separated by electrophoresis in 0.5× Tris-borate-EDTA buffer (Invitrogen, Carlsbad, CA) at 14°C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA) with pulse times of 2.16 to 63.8 s. Isolates presenting DNA smears were retested using plugs digested with XbaI and electrophoresis buffer containing 50 μM thiourea in 0.5× Tris-borate-EDTA buffer. The gels were stained with ethidium bromide, and DNA bands were visualized with UV transillumination (Bio-Rad).

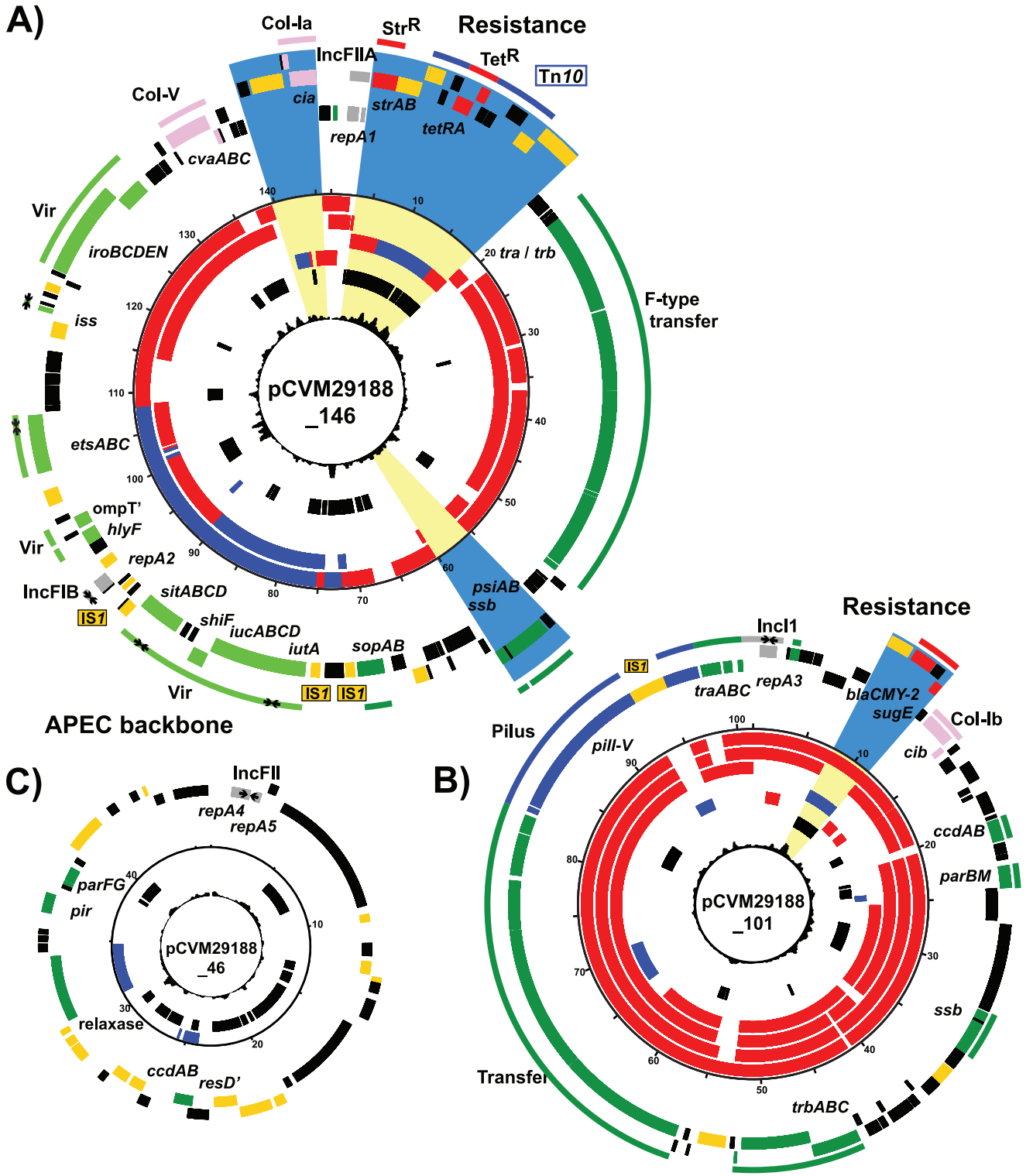


FIG. 1. Circular representation of the three large plasmids from *S.* Kentucky CVM29188. (A) pCVM29188_146. Circles, from outside to inside: 1 and 2, strand-specific depiction of all CDS, color coded according to predicted function in replication initiation (gray), antibiotic resistance (red), transposition/recombination (gold), plasmid transfer and maintenance (dark green), virulence (light green), colicin biosynthesis and immunity (pink), or other functions (black); 3, scale; 4 to 7, matching sequences (>95% nucleotide identity) in the same orientation (red) or opposite orientation (blue) with pAPEC-O1-ColBM, pAPEC-O2-ColV, pYR1, and pCVM29188_101; 8, predicted laterally transferred CDS (black); 9, trinucleotide sequence composition. (B) pCVM29188_101. Circles, from outside to inside: 1 and 2, strand-specific depiction of all CDS, color coded analogously to the circles in panel A, except for CDS encoding a conjugal pilus for liquid mating (blue); 3, scale; 4 to 8, matching sequences with pNF1358 from *S.* Typhimurium, ColIb-P9 from *Shigella sonnei* P9, R64 from *S.* Typhimurium, pSC138 from *S. enterica* Choleraesuis SC-B67, and pCVM29188_146; 9, predicted laterally transferred CDS (black); 10, trinucleotide sequence composition. (C) pCVM29188_46. Circles, from outside to inside: 1 and 2, strand-specific depiction of all CDS, color coded analogously to the circles in panels A and B; 3, scale; 4, matching sequences with pCVM29188_101; 5, predicted laterally transferred CDS (black); 6, trinucleotide sequence composition. PCR primer pair locations used for the APEC-like plasmid screening assay and for the verification of plasmid transfer experiments are shown on the outermost circles of all three plasmids.

PFGE results were analyzed using BioNumerics software (Applied Maths, Kortrijk, Belgium), and banding pattern similarities were compared using two-enzyme analysis with a 1.5% band position tolerance (29). All PFGE profiles on strains used in this study were submitted to the PulseNet national database located at the CDC for comparison with isolates from clinical salmonellosis cases.

Nucleotide sequence accession numbers. The plasmid sequences of the three *S.* Kentucky plasmids have been deposited in GenBank under accession numbers CP001122 (pCVM29188_146), CP001121 (pCVM29188_101), and CP001123 (pCVM29188_46).

RESULTS

Plasmid component of the *S.* Kentucky CVM29188 genome. The genome of *S.* Kentucky CVM29188 contains three large plasmids that have been designated pCVM29188_146 (146,811 bp), pCVM29188_101 (101,461 bp), and pCVM29188_46 (46,121 bp) with respect to their sizes (Fig. 1). The two largest plasmids carry resistance determinants that match the experimentally determined resistance phenotype of *S.* Kentucky CVM29188 to aminoglycosides (*strAB*), tetracyclines (*tetRA*), and cephalosporins (bla_{CMY-2}). The *strAB* and *tetRA* gene clusters were found on pCVM29188_146; bla_{CMY-2} and a gene for resistance to quaternary ammonium compounds (*sugE*) were found on pCVM29188_101. Resistance-conferring fragments on both plasmids also contain transposase genes and, on the basis of codon usage analysis (42), are predicted to originate from horizontal gene transfer (Fig. 1). The resistance fragment on pCVM29188_146 consists of the transposon Tn*10*, which is located next to two insertion (IS) elements (IS*1133* and the truncated element IS*903*), and integrated into Tn*5393* (see Fig. S1 in the supplemental material). It shows a composition similar to that of resistance islands on the resistance plasmids pYR1 from the fish pathogen *Yersinia ruckeri* (44) and on pEa34 from the fruit pathogen *Erwinia amylovora* (8). pCVM29188_101 carries a single composite transposon-like resistance element (*tnpA*-bla_{CMY-2}-*blc-sugE*), which is very similar to previously identified elements from salmonellae and other *Enterobacteriaceae* (37). pCVM29188_46 carries mostly hypothetical protein genes, none of which appear to contribute to the antimicrobial resistance phenotype of *S.* Kentucky CVM29188. Both pCVM29188_146 and pCVM29188_101 carry conjugative transfer systems of the type IV secretion system-like type. While the transfer region on pCVM29188_146 resembles the classic F-type plasmid originally found in *E. coli* (12), corresponding regions on pCVM29188_101 are more similar to the plasmids R64 from *Salmonella enterica* subsp. *enterica* serovar Typhimurium and ColIb-P9 from *Shigella sonnei* P9 (35) and contain the *pil* operon that encodes a thin conjugal pilus required for liquid mating (19a). The smallest plasmid, pCVM29188_46, does not carry an independent transfer region. pCVM29188_146 carries two origins of replication, belonging to incompatibility groups IncFIB and IncFIIA. The *repA* gene on pCVM29188_101 belongs to incompatibility group IncI1. Another IncFII origin of replication is found on pCVM29188_46.

Comparative plasmid analysis. Comparative sequence analysis of pCVM29188_146 with other completely sequenced plasmids from the NCBI database identified a highly conserved, largely syntenic plasmid backbone of about 106 kb, which is shared between pCVM29188_146 and two APEC virulence plasmids, pAPEC-O1-ColBM (17) and pAPEC-O2-ColV (18) (Fig. 1). Overall gene synteny between the three plasmids is interrupted at only a few sites that in many cases are characterized by the presence of IS elements (Fig. 1). The conjugative transfer region, the two replicative origins, and a putative pathogenicity island (PAI) (18) are all located within the conserved plasmid backbone. APEC virulence factors located within the PAI include iron acquisition and transport systems, such as the aerobactin siderophore system, encoded by *iutA iucABCD* (9), and the salmochelin siderophore system, encoded by *iroBCDEN* (14). Also found within this region were an ABC transport system encoded by *etsABC* (18) and an iron/manganese transport system encoded by *sitABCD* (32). The genes encoding the increased serum survival protein Iss (4), the putative hemolysin HlyF (26), and a truncated outer membrane protease, OmpT′ (30), are located within the same PAI and have also been associated with APEC virulence phenotypes.

pCVM29188_146 carries two gene clusters for colicin biosynthesis, export, and immunity. Only the colicin-V gene cluster (*cvaABC*) is shared with one of the other two sequenced APEC plasmids (pAPEC-O2-ColV); a second gene cluster encodes the proteins for biosynthesis of and immunity to colicin-Ia. Colicin-V and colicin-Ia appear to be genetically linked, as they often occur together in single strains (13) and on the same conjugative plasmids (16). Codon usage and trinucleotide sequence composition analysis of pCVM29188_146 suggest that the colicin-V operon, but not the colicin-Ia operon, was most likely acquired through lateral gene transfer (Fig. 1).

The second-largest plasmid from the *S.* Kentucky CVM29188 genome, pCVM29188_101, is very similar (>80% nucleotide sequence identity) to the *S.* Typhimurium plasmid pNF1358 (95% coverage) and to ColIb-P9 from *Shigella sonnei* P9 (89% coverage), except for a copy of IS*1* that is absent from the other two plasmids (Fig. 1). IS*1* is the only IS element that is present both on pCVM29188_146 (three copies) and pCVM29188_101 (one copy). The *tnpA*-bla_{CMY-2}-*blc-sugE* resistance gene cassette on pCVM29188_101 is also present on pNF1358 but is absent from ColIb-P9. pCVM29188_101 carries the third colicin gene cluster, found within the *S.* Kentucky CVM29188 genome, which encodes proteins for colicin-Ib synthesis (*cib*) and immunity.

The smallest plasmid, pCVM29188_46, shows little similarity to previously sequenced plasmids. Several protein-coding sequences (CDS) on pCVM29188_46 are also found on pCVM29188_101. These include genes for putative components of the type IV conjugative relaxosome and for a toxin-antitoxin module (*ccdAB*).

Conjugative plasmid transfer. To measure conjugative plasmid transfer, *S.* Kentucky CVM29188 was used as a plasmid donor, and Rif-resistant derivatives of the plasmid-free strains *S.* Kentucky CVM35942, *S.* Newport SL317, and *E. coli* DH10B were used as plasmid recipients. Tetracycline and ceftiofur resistance phenotypes, encoded by pCVM29188_146 and pCVM29188_101, respectively, were used as selection markers for transfer of both plasmids individually and jointly (Fig. 2). By use of this system, transfer of both plasmids, alone and combined, to each of the three recipient strains could be detected. Transfer of pCVM29188_101 alone to each of the recipients (8.9×10^{-2} to 1.4×10^{-1} transconjugants/CFU of donor) was more efficient than that of pCVM29188_146 alone

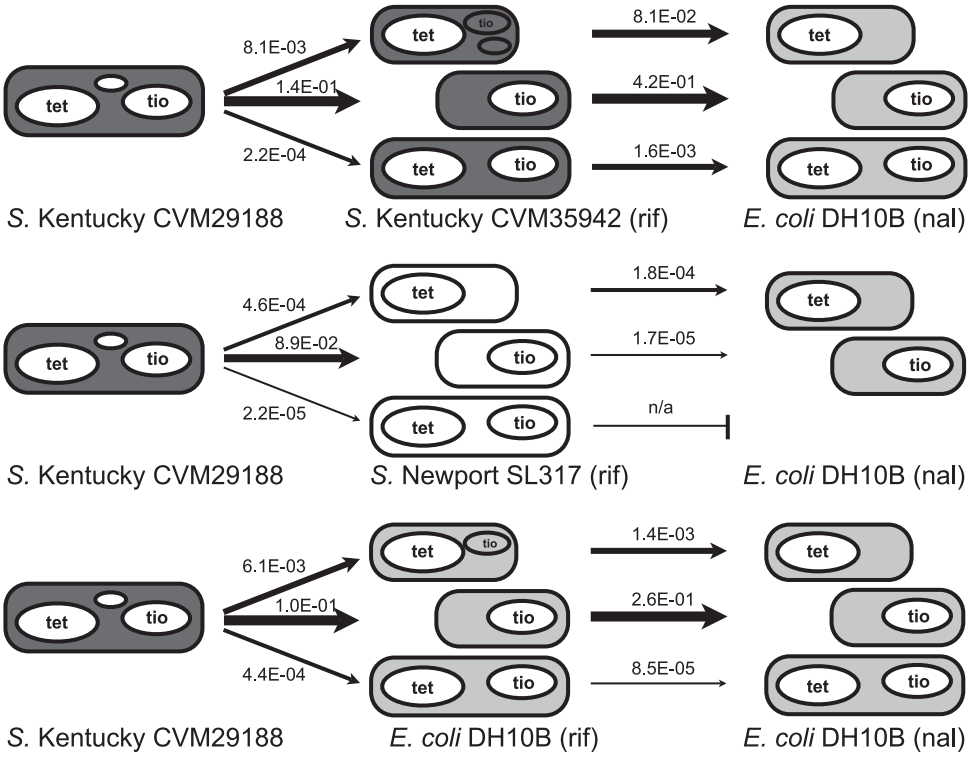
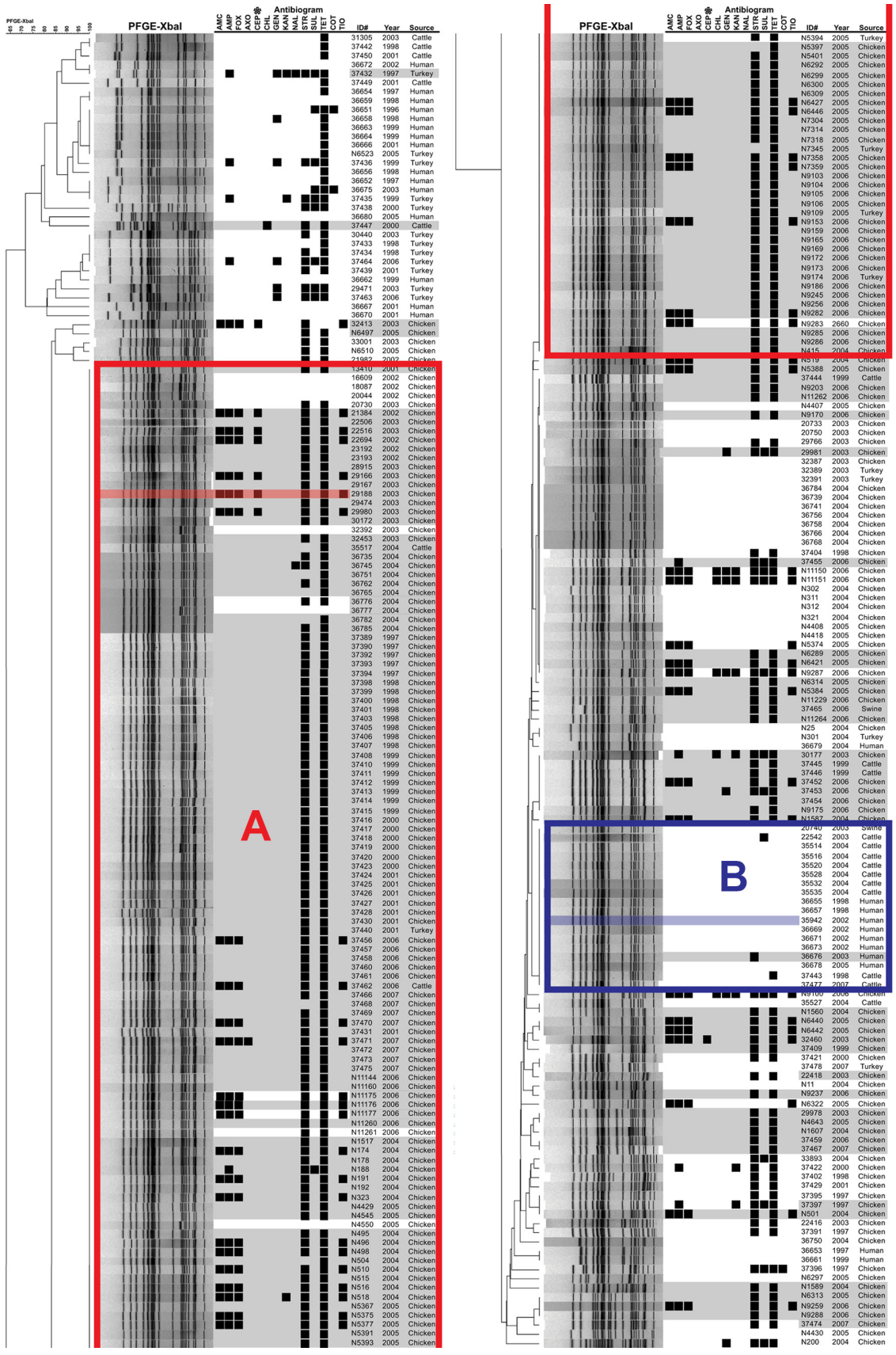


FIG. 2. Schematic representation of the conjugative plasmid transfer system set up between *S.* Kentucky CVM29188 (dark gray) as a plasmid donor and *S.* Kentucky CVM35942 (dark gray), *S.* Newport SL317 (white), and *E. coli* DH10B (light gray) as plasmid recipients. Transconjugants from the first plasmid transfer served as plasmid donors in the retransfer to *E. coli* DH10B (light gray). Transfer efficiencies are given as numbers of transconjugants per cell-forming unit of donor. Where detected, cotransfer is depicted through plasmid circles without fillings. tet, tetracycline; tio, ceftiofur.

(4.6×10^{-4} to 8.1×10^{-3} transconjugants/CFU) or of both plasmids combined (2.2×10^{-5} to 4.4×10^{-4} transconjugants/CFU). Transfer efficiencies were dependent on recipient strains, with both *S.* Kentucky CVM35942 and *E. coli* DH10B being ~10-fold more efficient than the *S.* Newport strain under all three conditions tested (Fig. 2). Randomly chosen transconjugants obtained under selective conditions for either pCVM29188_146 (tetracycline) or pCVM29188_101 (ceftiofur) alone were also streaked on agar plates containing the corresponding other antimicrobial not used for the mating experiment. These phenotype assays showed that four transconjugants (three *S.* Kentucky strain and one *E. coli* strain) out of 78 clones tested (13 per recipient and antimicrobial compound), in addition to the tetracycline resistance phenotype used for selection of pCVM29188_146, also exhibited ceftiofur resistance. Cotransfer of tetracycline resistance (pCVM29199_146) in transconjugants obtained through selection for ceftiofur resistance (pCVM29188_101) was not detected in our experiments. PCR screenings with plasmid-specific primer pairs confirmed that, except for the four clones with both resistance phenotypes, all transconjugants contained only either pCVM29188_146 or pCVM29188_101. Of the four transconjugants exhibiting both resistance phenotypes, the single *E. coli* strain tested positive for the presence of both pCVM29188_146 and pCVM29188_101, whereas the three *S.* Kentucky strains contained all three plasmids from *S.* Kentucky CVM29188.

To determine whether both pCVM29188_146 and pCVM29188_101 were self-transferable or whether transfer was mediated by only one of the two plasmids, retransfer experiments were performed. Rif-resistant transconjugants from the first mating were used as plasmid donors, and a Nal-resistant plasmid-free *E. coli* DH10B derivative was used as the recipient strain (Fig. 2). In these experiments, both plasmids were self-transferable from transconjugants containing only either pCVM29188_146 or pCVM29188_101, independently of the respective host strain, showing that the transfer machineries on each of the two plasmids were sufficient for conjugative transfer. In the case of pVM29188_146, the efficiencies for this retransfer from all transconjugants to the *E. coli* recipient were comparable to or even higher than those for the first plasmid transfer experiments (Fig. 2). However, while the retransfer efficiencies of pCVM29188_101 alone and together with pCVM29188_146 from *S.* Kentucky and *E. coli* transconjugants to *E. coli* were also comparable to those for the first mating experiments, retransfer of pCVM29188_101 from *S.* Newport transconjugants to *E. coli* was significantly reduced (factor, 2×10^{-4}) and retransfer of both plasmids together even below the detectable threshold of 3.2×10^{-6}.

Prevalence of APEC-like plasmids in *S.* Kentucky. With a set of five PCR primer pairs specific for pCVM29188_146 (binding to *repA*^{FIB}, *sitABCD*, *iutA iucD*, *iss*, and *etsABC*) (see Table S1 in the supplemental material), *S.* Kentucky isolates from meat, agricultural, and clinical sources, as well as several


PFGE-XbaI
Antibiogram
AMC AMP FOX AXO CEP CHL GEN KAN NAL STR SUL TET COT TIO
ID# Year Source
A
B

other nontyphoidal *Salmonella* serovar isolates, were screened for the presence of APEC-like plasmids (see Table S2 in the supplemental material). In these screening assays, 287 *S.* Kentucky strains, isolated between 1997 and 2006 and mainly from chicken (221 isolates) samples, but also from turkey (17 isolates), cattle (21 isolates), swine (2 isolates), and human (26 isolates) samples, were examined. Veterinary and retail meat isolates from the same animal source showed similar screening profiles (see Table S2 in the supplemental material for comparison of chicken and chicken meat samples). Therefore, no distinction will be made in the following results between animal and animal meat isolates. The complete screening results, including information about sample sources, are presented in Table S2 in the supplemental material. Out of 287 *S.* Kentucky strains, 183 (64%) tested positive for the presence of at least one locus and 173 (60%) for the presence of all five plasmid loci. When only the *S.* Kentucky isolates from chicken samples were examined, 164 (74%) tested positive for all APEC-like plasmid loci, with annual percentages ranging between 50% in 2002 (four of eight isolates) and 100% in 1999 (eight of eight isolates) (see Table S2 in the supplemental material). APEC-like plasmids were identified in *S.* Kentucky isolates from each source tested, but fractions in *S.* Kentucky from other sources were significantly lower than those found in the chicken isolates, varying from 0.06% (1/18 turkey isolates) to 0.04% (1/26 human isolates), 29% (6/21 cattle isolates), and 50% (1/2 swine isolates). Three turkey isolates tested positive for one (*iss*) plasmid locus, two (*iutA iucD* and *sitABCD*) plasmid loci, and three (*iutA iucD*, *sitABCD*, and *repA*^{FIB}) plasmid loci. Apart from *S.* Kentucky, 60 *Salmonella* isolates from chicken sources belonging to six other serovars were tested (see Table S2 in the supplemental material): *S.* Typhimurium (24 isolates), *S.* Typhimurium var. O5- (10 isolates), *Salmonella enterica* subsp. *enterica* serovar Enteritidis (9 isolates), *Salmonella enterica* subsp. *enterica* serovar Heidelberg (15 isolates), *Salmonella enterica* subsp. *enterica* serovar I4,12:i:- (1 isolate), and *S.* I4,5,12:i:- (1 isolate). With the exception of two *S.* Typhimurium var. O5- strains that tested positive for three of the plasmid loci (*repA*^{FIB}, *sitABCD*, and *iutA iucD*), all strains tested negative.

All screened *S.* Kentucky isolates were also characterized with respect to antimicrobial susceptibility and XbaI digestion PFGE patterns (Fig. 3). Clustering based on XbaI PFGE pattern diversity placed most *S.* Kentucky isolates from chickens into a single indistinguishable pattern (Fig. 3, group A) that is populated only sparsely with strains from other sources and does not contain a single human isolate. The vast majority of strains containing APEC-like plasmids fell into this indistinguishable pattern. Except for the single human isolate, all *S.* Kentucky strains carrying APEC-like plasmids show resistance to tetracycline and, with the exception of nine other strains, resistance to streptomycin, which is in accordance with the finding that in *S.* Kentucky CVM29188, streptomycin and tetracycline resistances are mediated by CVM29188_146. Ceftiofur resistance, mediated by pCVM29188_101 in *S.* Kentucky CVM29188, was found in 38 (22%) of the *S.* Kentucky strains that tested positive for the presence of APEC-like plasmids, compared to only 10 (9%) of all the remaining isolates with this resistance phenotype. Several of the human isolates exhibited indistinguishable XbaI PFGE patterns with two cattle isolates and one swine isolate (Fig. 3, group B). The single human *S.* Kentucky isolate that tested positive for the presence of APEC-like plasmids belongs to this group; all other isolates from this group tested negative.

DISCUSSION

In this work, we demonstrate that antimicrobial drug resistance in *S.* Kentucky CVM29188 is mediated by two of three large plasmids. Resistance phenotypes were transferable to susceptible *S.* Kentucky, *S.* Newport, and *E. coli* strains, demonstrating that both pCVM29188_146 and pCVM29188_101, alone and combined, have the potential for conjugative transfer across both serovar and species boundaries. Transfer assays showed that tetracycline resistance is conferred by pCVM29188_146 and encoded as part of a larger, Tn*10*-containing resistance fragment with similarity to drug resistance plasmids from *Yersinia ruckeri* (44) and *Erwinia amylovora* (8). A Tn*5393* transposon within the same resistance fragment carries the *strAB* gene cluster for streptomycin resistance. A transposon-like resistance element with wide distribution among salmonellae and other *Enterobacteriaceae* (37) is responsible for ceftiofur resistance mediated by pCVM29188_101. The high degree of sequence conservation of these resistance elements from unrelated plasmids from different bacterial hosts supports the concept that a shared horizontal resistance gene pool is available to diverse bacterial communities from environmental, agricultural, and clinical settings (11). The annotation of pCVM29188_46 suggests that the smallest plasmid is not involved in the antimicrobial resistance phenotype of *S.* Kentucky CVM29188.

Comparative sequence analysis suggests that both pCVM29188_146 and pCVM29188_101 evolved from common plasmid backbones by acquisition and integration of horizontally transferred resistance gene cassettes into larger mosaic plasmid resistance islands. Notably, the backbone of pCVM29188_146 bears strong resemblance to a group of APEC virulence plasmids, previously found only in *E. coli* and thought responsible for causing colibacillosis in chickens (17, 18). Acquisition of one of these two plasmids, pAPEC-O2-ColV, by commensal *E. coli* strains has been shown to result in increased abilities to kill avian embryos, grow in human urine,

FIG. 3. PFGE dendrogram including antimicrobial resistance spectrum of *S.* Kentucky isolates screened for the presence of APEC-like plasmids. Strains testing positive for the presence of all five plasmid marker loci are shaded in gray on entire lines. Strains testing positive for only a subset of the plasmid marker loci are shaded gray only partially. Two large groups of isolates indistinguishable by XbaI restriction are highlighted: A, which contains mostly chicken *S.* Kentucky isolates (94%), and B, which contains human and cattle isolates, but without chicken *S.* Kentucky isolates. Abbreviations for antimicrobials: AMC, amoxicillin (amoxicilline)-clavulanic acid; AMP, ampicillin; FOX, cefoxitin; AXO, ceftriaxone; CEP*, cephalothin (cefalotin) (only tested for NARMS isolates from 2002 to 2003); CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; STR, streptomycin; SUL, sulfamethoxazole; TET, tetracycline; COT, trimethoprim-sulfamethoxazole; TIO, ceftiofur.

and colonize the murine kidney (36). Shared sequence regions encode putative virulence factors involved in avian pathogenesis (5, 31, 33, 39), some of which may also play a role in uropathogenic *E. coli* infections in humans (30, 36). APEC-like resistance plasmids similar to pCVM29188_146 were also found in a group of *S.* Kentucky isolates that, according to PFGE-based phylogenetic predictions, constitute a highly related group of *S.* Kentucky strains that is predominant in chicken. Given the shared intestinal habitat, it is likely that *S.* Kentucky acquired APEC-like plasmids from commensal and/or pathogenic *E. coli* strains in the chicken intestine. To our knowledge, transfer of virulence genes between *E. coli* and *Salmonella* on mobile genetic elements has previously been shown only once, in the case of the heat-stable toxin gene *astA*, which is likely to have been acquired by a gifsy-2-related prophage in *Salmonella enterica* subsp. *enterica* serovar Abortusovis from a pathogenic *E. coli* strain (3).

In 94% of all tested *S.* Kentucky chicken strains, the presence of APEC-like plasmids was associated with two different antimicrobial resistance phenotypes, tetracycline and streptomycin. Both resistance phenotypes could be encoded by APEC-like resistance plasmids similar in composition to pCVM29188_146. In this context, it is noteworthy that within the last decade the prevalence of *S.* Kentucky in chickens, compared to that of other *Salmonella* serovars, has constantly been increasing (40) and that tetracycline and streptomycin resistances represent the two most common resistance phenotypes in *S.* Kentucky from chickens (10). Together, these results indicate the possibility of a newly emerging *S.* Kentucky lineage with APEC-like resistance plasmids similar to pCVM29188_146 in chickens.

The physical linkage of genetic determinants for both antimicrobial resistance and APEC virulence on the same plasmid, as seen for pCVM29188_146, allows for at least two different evolutionary models to explain the wide distribution of similar plasmids in *S.* Kentucky chicken populations: the spread of APEC-like plasmids in *S.* Kentucky from chickens could have been facilitated through the selection for either antimicrobial resistance or phenotypes associated with putative APEC virulence factors. In both cases, a positive selection in the Darwinian sense would describe the results of a discrimination within the *S.* Kentucky populations against strains lacking APEC-like plasmids. In the first scenario, antimicrobial selection could have promoted the integration of resistance gene cassettes into APEC virulence plasmids from pathogenic *E. coli* strains. In a second step, mediated through antimicrobial selection, APEC-derived resistance plasmids could have been mobilized within enterobacterial communities, leading to their presence in *S.* Kentucky strains. Tetracycline is used in chicken production to treat secondary *E. coli* infections in poultry (25). According to this scenario, similar APEC-like resistance plasmids should also be present in other enterobacteria from the same environment and also from other settings under similar antimicrobial selection. In this study, however, the majority of APEC-like plasmids were identified in *S.* Kentucky isolates from chicken but rarely in other *Salmonella* serovars from chicken or in *S.* Kentucky isolates from other sources.

In the second scenario, the prevalence of APEC-like plasmids in *S.* Kentucky strains from chicken could result from selective benefits that these plasmids confer to their host in the colonization of the chicken gut. Infection with *S.* Kentucky is usually not associated with disease patterns in chicken (John Maurer, personal communication). However, plasmid-encoded APEC virulence factors have been associated with resistance to serum (*iss*) (22, 23) and oxidative stress (*sitABCD*) (33, 34) as well as with the biosynthesis of the siderophores salmochelin (14) and aerobactin (9) and other transport mechanisms, such as those encoded by *sitABCD* (32) and *etsABC* (18). These APEC virulence factors could provide *S.* Kentucky strains with selective advantages in coping with stress factors associated with the intestinal environment or in competing with other enterobacteria for limited resources.

The results presented in this study show that antimicrobial resistance determinants and APEC virulence factors implied in avian and possibly human pathogenesis can be encoded by the same plasmid. Under antimicrobial selection, the propagation of these virulence factors within bacterial communities could potentially lead to the emergence of new virulent strains from the commensal microflora. We therefore suggest that future sequencing studies focus on plasmid population dynamics within host-associated microbiota in order to examine the prevalence and spread of combined virulence and resistance plasmids in bacteria from agricultural, environmental, and clinical settings. Horizontal transfer of plasmids not directly selected for by a given antimicrobial agent, as seen for the ceftiofur resistance plasmid pCVM29188_101 in conjugation assays selecting for the tetracycline resistance plasmid pCVM29188_146, is another area worthy of exploration and may be important to consider in addition to cotransfer of resistance genes linked on the plasmid under direct selection.

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REFERENCES

1. **Aarestrup, F. M., R. S. Hendriksen, J. Lockett, K. Gay, K. Teates, P. F. McDermott, D. G. White, H. Hasman, G. Sorensen, A. Bangtrakulnonth, S. Pornreongwong, C. Pulsrikarn, F. J. Angulo, and P. Gerner-Smidt.** 2007. International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. Emerg. Infect. Dis. **13:**726–731.
2. **Angulo, F. J., V. N. Nargund, and T. C. Chiller.** 2004. Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J. Vet. Med. B **51:**374–379.
3. **Bacciu, D., G. Falchi, A. Spazziani, L. Bossi, G. Marogna, G. S. Leori, S. Rubino, and S. Uzzau.** 2004. Transposition of the heat-stable toxin *astA* gene into a gifsy-2-related prophage of *Salmonella enterica* serovar Abortusovis. J. Bacteriol. **186:**4568–4574.
4. **Binns, M. M., D. L. Davies, and K. G. Hardy.** 1979. Cloned fragments of the plasmid ColV,I-K94 specifying virulence and serum resistance. Nature **279:** 778–781.
5. **Caza, M., F. Lepine, S. Milot, and C. M. Dozois.** 2008. Specific roles of the *iroBCDEN* genes in virulence of an avian pathogenic *Escherichia coli* O78 strain and in production of salmochelins. Infect. Immun. **76:**3539–3549.
6. **CDC.** 2008. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS). Human isolates final report, 2005. CDC, Atlanta, GA. http://www.cdc.gov/narms/annual/2005/NARMSAnnualReport2005.pdf.
7. **CDC.** 2008. Public Health Laboratory Information System (PHLIS). *Salmonella* surveillance, annual summary, 2006. CDC, Atlanta, GA. http://www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2006/SalmonellaAnnualSummary2006.pdf.
8. **Chiou, C. S., and A. L. Jones.** 1993. Nucleotide sequence analysis of a transposon (Tn*5393*) carrying streptomycin resistance genes in *Erwinia amylovora* and other gram-negative bacteria. J. Bacteriol. **175:**732–740.
9. **de Lorenzo, V., and J. B. Neilands.** 1986. Characterization of *iucA* and *iucC*

genes of the aerobactin system of plasmid ColV-K30 in *Escherichia coli*. J. Bacteriol. **167:**350–355.
10. **FDA.** 2008. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS). Retail meat annual report, 2006. FDA, Bethesda MD. http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM073302.pdf.
11. **Fricke, W. F., M. S. Wright, A. H. Lindell, D. M. Harkins, C. Baker-Austin, J. Ravel, and R. Stepanauskas.** 2008. Insights into the environmental resistance gene pool from the genome sequence of the multidrug-resistant environmental isolate *Escherichia coli* SMS-3-5. J. Bacteriol. **190:**6779–6794.
12. **Frost, L. S., K. Ippen-Ihler, and R. A. Skurray.** 1994. Analysis of the sequence and gene products of the transfer region of the F sex factor. Microbiol. Rev. **58:**162–210.
13. **Gordon, D. M., and C. L. O'Brien.** 2006. Bacteriocin diversity and the frequency of multiple bacteriocin production in *Escherichia coli*. Microbiology **152:**3239–3244.
14. **Hantke, K., G. Nicholson, W. Rabsch, and G. Winkelmann.** 2003. Salmochelins, siderophores of *Salmonella enterica* and uropathogenic *Escherichia coli* strains, are recognized by the outer membrane receptor IroN. Proc. Natl. Acad. Sci. USA **100:**3677–3682.
15. **Jensen, V. F., J. Neimann, A. M. Hammerum, K. Molbak, and H. C. Wegener.** 2004. Does the use of antibiotics in food animals pose a risk to human health? An unbiased review? J. Antimicrob. Chemother. **54:**274–278.
16. **Jeziorowski, A., and D. M. Gordon.** 2007. Evolution of microcin V and colicin Ia plasmids in *Escherichia coli*. J. Bacteriol. **189:**7045–7052.
17. **Johnson, T. J., S. J. Johnson, and L. K. Nolan.** 2006. Complete DNA sequence of a ColBM plasmid from avian pathogenic *Escherichia coli* suggests that it evolved from closely related ColV virulence plasmids. J. Bacteriol. **188:**5975–5983.
18. **Johnson, T. J., K. E. Siek, S. J. Johnson, and L. K. Nolan.** 2006. DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. J. Bacteriol. **188:**745–758.
19. **Karp, B. E., and J. Engberg.** 2004. Comment on: does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. **54:**273–278.
19a. **Kim, S. R., and T. Komano.** 1997. The plasmid R64 thin pilus identified as a type IV pilus. J. Bacteriol. **179:**3594–3603.
20. **Kurtz, S., A. Phillippy, A. L. Delcher, M. Smoot, M. Shumway, C. Antonescu, and S. L. Salzberg.** 2004. Versatile and open software for comparing large genomes. Genome Biol. **5:**R12.
21. **Layton, A. N., and E. E. Galyov.** 2007. *Salmonella*-induced enteritis: molecular pathogenesis and therapeutic implications. Expert Rev. Mol. Med. **9**(18)**:**1–17.
22. **Lynne, A. M., J. A. Skyberg, C. M. Logue, C. Doetkott, S. L. Foley, and L. K. Nolan.** 2007. Characterization of a series of transconjugant mutants of an avian pathogenic *Escherichia coli* isolate for resistance to serum complement. Avian Dis. **51:**771–776.
23. **Lynne, A. M., J. A. Skyberg, C. M. Logue, and L. K. Nolan.** 2007. Detection of Iss and Bor on the surface of *Escherichia coli*. J. Appl. Microbiol. **102:**660–666.
24. **McDermott, P. F., S. Zhao, D. D. Wagner, S. Simjee, R. D. Walker, and D. G. White.** 2002. The food safety perspective of antibiotic resistance. Anim. Biotechnol. **13:**71–84.
25. **McEwen, S. A., and P. J. Fedorka-Cray.** 2002. Antimicrobial use and resistance in animals. Clin. Infect. Dis. **34**(Suppl. 3)**:**S93–S106.
26. **Morales, C., M. D. Lee, C. Hofacre, and J. J. Maurer.** 2004. Detection of a novel virulence gene and a *Salmonella* virulence homologue among *Escherichia coli* isolated from broiler chickens. Foodborne Pathog. Dis. **1:**160–165.
27. **Phillips, I., M. Casewell, T. Cox, B. De Groot, C. Friis, R. Jones, C. Nightingale, R. Preston, and J. Waddell.** 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. **53:**28–52.
28. **Read, T. D., S. N. Peterson, N. Tourasse, L. W. Baillie, I. T. Paulsen, K. E. Nelson, H. Tettelin, D. E. Fouts, J. A. Eisen, S. R. Gill, E. K. Holtzapple, O. A. Okstad, E. Helgason, J. Rilstone, M. Wu, J. F. Kolonay, M. J. Beanan, R. J. Dodson, L. M. Brinkac, M. Gwinn, R. T. DeBoy, R. Madpu, S. C. Daugherty, A. S. Durkin, D. H. Haft, W. C. Nelson, J. D. Peterson, M. Pop, H. M. Khouri, D. Radune, J. L. Benton, Y. Mahamoud, L. Jiang, I. R. Hance, J. F. Weidman, K. J. Berry, R. D. Plaut, A. M. Wolf, K. L. Watkins, W. C. Nierman, A. Hazen, R. Cline, C. Redmond, J. E. Thwaite, O. White, S. L. Salzberg, B. Thomason, A. M. Friedlander, T. M. Koehler, P. C. Hanna, A. B. Kolsto, and C. M. Fraser.** 2003. The genome sequence of *Bacillus anthracis* Ames and comparison to closely related bacteria. Nature **423:**81–86.
29. **Ribot, E. M., M. A. Fair, R. Gautom, D. N. Cameron, S. B. Hunter, B. Swaminathan, and T. J. Barrett.** 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli O157:H7*, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog. Dis. **3:**59–67.
30. **Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, M. K. Fakhr, and L. K. Nolan.** 2005. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology **151:**2097–2110.
31. **Rodriguez-Siek, K. E., C. W. Giddings, C. Doetkott, T. J. Johnson, and L. K. Nolan.** 2005. Characterizing the APEC pathotype. Vet. Res. **36:**241–256.
32. **Runyen-Janecky, L. J., S. A. Reeves, E. G. Gonzales, and S. M. Payne.** 2003. Contribution of the *Shigella* flexneri Sit, Iuc, and Feo iron acquisition systems to iron acquisition in vitro and in cultured cells. Infect. Immun. **71:**1919–1928.
33. **Sabri, M., M. Caza, J. Proulx, M. H. Lymberopoulos, A. Bree, M. Moulin-Schouleur, R. Curtiss III, and C. M. Dozois.** 2008. Contribution of the SitABCD, MntH, and FeoB metal transporters to the virulence of avian pathogenic *Escherichia coli* O78 strain chi7122. Infect. Immun. **76:**601–611.
34. **Sabri, M., S. Leveille, and C. M. Dozois.** 2006. A SitABCD homologue from an avian pathogenic *Escherichia coli* strain mediates transport of iron and manganese and resistance to hydrogen peroxide. Microbiology **152:**745–758.
35. **Sakai, D., and T. Komano.** 2002. Genes required for plasmid R64 thin-pilus biogenesis: identification and localization of products of the *pilK*, *pilM*, *pilO*, *pilP*, *pilR*, and *pilT* genes. J. Bacteriol. **184:**444–451.
36. **Skyberg, J. A., T. J. Johnson, J. R. Johnson, C. Clabots, C. M. Logue, and L. K. Nolan.** 2006. Acquisition of avian pathogenic *Escherichia coli* plasmids by a commensal *E. coli* isolate enhances its abilities to kill chicken embryos, grow in human urine, and colonize the murine kidney. Infect. Immun. **74:**6287–6292.
37. **Su, L. H., H. L. Chen, J. H. Chia, S. Y. Liu, C. Chu, T. L. Wu, and C. H. Chiu.** 2006. Distribution of a transposon-like element carrying *bla*(*CMY-2*) among *Salmonella* and other Enterobacteriaceae. J. Antimicrob. Chemother. **57:**424–429.
38. **Tenover, F. C.** 24 June 2008, posting date. CDC's role in monitoring and preventing antimicrobial resistance. CDC, Atlanta, GA. http://help.senate.gov/Hearings/2008_06_24/Tenover.pdf.
39. **Tivendale, K. A., J. L. Allen, C. A. Ginns, B. S. Crabb, and G. F. Browning.** 2004. Association of iss and *iucA*, but not *tsh*, with plasmid-mediated virulence of avian pathogenic *Escherichia coli*. Infect. Immun. **72:**6554–6560.
40. **USDA.** 2008. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS). Veterinary isolates final report, slaughter isolates, 2006. USDA, Washington, DC. http://www.ars.usda.gov/sp2UserFiles/Place/66120508/NARMS/narms_2006/NARMS2006.pdf.
41. **Voetsch, A. C., T. J. Van Gilder, F. J. Angulo, M. M. Farley, S. Shallow, R. Marcus, P. R. Cieslak, V. C. Deneen, and R. V. Tauxe.** 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin. Infect. Dis. **38**(Suppl. 3)**:**S127–S134.
42. **Waack, S., O. Keller, R. Asper, T. Brodag, C. Damm, W. F. Fricke, K. Surovcik, P. Meinicke, and R. Merkl.** 2006. Score-based prediction of genomic islands in prokaryotic genomes using hidden Markov models. BMC Bioinformatics **7:**142.
43. **Wassenaar, T. M.** 2005. Use of antimicrobial agents in veterinary medicine and implications for human health. Crit. Rev. Microbiol. **31:**155–169.
44. **Welch, T. J., W. F. Fricke, P. F. McDermott, D. G. White, M. L. Rosso, D. A. Rasko, M. K. Mammel, M. Eppinger, M. J. Rosovitz, D. Wagner, L. Rahalison, J. E. Leclerc, J. M. Hinshaw, L. E. Lindler, T. A. Cebula, E. Carniel, and J. Ravel.** 2007. Multiple antimicrobial resistance in plague: an emerging public health risk. PLoS ONE **2:**e309.
45. **White, D. G., S. Zhao, P. F. McDermott, S. Ayers, S. Gaines, S. Friedman, D. D. Wagner, J. Meng, D. Needle, M. Davis, and C. DebRoy.** 2002. Characterization of antimicrobial resistance among *Escherichia coli* O111 isolates of animal and human origin. Microb. Drug Resist. **8:**139–146.